THE ECOLOGY OF TOBACCO THRIPS, *FRANKLINIELLA FUSCA*, OCCURRING ON PEANUT: I. NATURAL ENEMIES. II. DEVELOPMENTAL BIOLOGY IN ASSOCIATION WITH TOMATO SPOTTED WILT VIRUS

By

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS....ii

LIST OF TABLES....v

LIST OF FIGURES....vi

ABSTRACT....vii

1 INTRODUCTION....1

2 DEVELOPMENT OF TOBACCO THRIPS, *FRANKLINIELLA FUSCA*, ON THREE HOSTS INCLUDING PEANUT INFECTED WITH TOMATO SPOTTED WILT TOSPOVIRUS....5
  - Introduction....5
  - Materials and Methods....7
    - Host Developmental Study....7
    - Viral Infection on Developmental Rates....9
    - Leaf Nitrogen Content....9
  - Results....10
    - Host Developmental Study....10
    - Leaf Nitrogen Study....12
    - Viral Infection on Developmental Rates....12
  - Discussion....15

3 NATURAL ENEMIES OF *FRANKLINIELLA FUSCA*....20
  - Introduction....20
  - Materials and Methods....21
  - Results....22
  - Discussion....27

4 *THRIPINEMA FUSCUM* N. SP. (TYLENCHIDA: ALLANTONEMATIDAE), A PARASITE OF THE TOBACCO THRIPS, *FRANKLINIELLA FUSCA* (THYSANOPTERA)........31
Introduction........31
Materials and Methods........32
    Collection of Nematodes........32
    Light Microscopy Preparation........33
    Systematics........33
    Description........33
    Type Host and Locality........37
    Type Specimens........37
    Relationships........38
    Key to the Species of the Genus *Thripinema*........38
    Biology........39

REFERENCES........41

BIOGRAPHICAL SKETCH........51

LIST OF TABLES

Table page

2.1: Mean developmental times (days) of first and second instar larvae and pupae of*Frankliniella fusca* (±SEM) and larval mortality on three hosts at 25°C. 11

2.2: Chi-square analysis of mortality percentages for *Frankliniella fusca* on infected and healthy peanut. 13

2.3: Mean development times (days) on peanut of larvae and pupae of *Frankliniella fusca* (±SEM) infected or non-infected with tomato spotted wilt tospovirus at 25°C. 14

3.1: Percentage of parasitism of *Frankliniella fusca* by *Thripinema fuscum* in peanut from Marianna, Florida 1995 (average = 49.8) 28

3.2: Percentage of parasitism of *Frankliniella fusca* by *Thripinema fuscum* in peanut from Marianna, Florida 1996 (average = 40.8). 29

3.3: Percentage of parasitism of *Frankliniella fusca* by *Thripinema fuscum* in peanut from Marianna, Florida 1997 (average = 47.8). 30

LIST OF FIGURES

Figure page

3.1: Vial trap. 23

3.2: Number of thrips per sample of *Frankliniella fusca* stages collected in peanut, *Arachis hypogaea* during summer 1995, Marianna, Florida. 24

3.3: Number of thrips per sample of *Frankliniella fusca* stages collected in peanut, *Arachis hypogaea* during summer 1996, Marianna, Florida. 25

3.4: Number of thrips per sample of *Frankliniella fusca* stages collected inpeanut, *Arachis hypogaea* during summer 1997, Marianna, Florida. 26

4.1: *Thripinema fuscum* n. sp. female. A) Infective female. B) Anterior region of an infective female. C, D, F) Progressive enlargement of parasitic females. E) Gonad of infective female. Scale bar 26 µm for A, C, D, F; 12 µm for B, E. 34

4.2: *Thripinema fuscum* n. sp. male. A) Anterior region of male. B) Young male exhibiting slight dorsal curve. C) Bursa. D) Lateral field of male. Scale bar 14 µm for A, C, D; 30 µm for B. 35

Abstract of Dissertation Presented to the Graduate School of the
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Few natural enemies have been documented for tobacco thrips, *Frankliniella fusca* (Hinds). Examination and samples of field populations discovered that a nematode parasite, *Thripinema fuscum* Tipping and Nguyen n. sp., is an important regulator of populations of *F. fusca* in peanut fields in north Florida, with parasitism rates between 40-70 percent during the growing season.

The developmental rates of *F. fusca* was examined on three hosts: peanut (*Arachis hypogaea* L.), cotton (*Gossypium hirsutum* L.), and tomato (*Lycopersicon esculentum* L.). Peanut and cotton appeared to be ideal hosts for *F.* fusca, with similar developmental times for these two hosts. Tomato is not a good host for *F. fusca*, with developmental times significantly slower than peanut or cotton.

Frankliniella fusca is also an important vector of tomato spotted wilt tospovirus in peanut and other crops. This virus appeared to have an effect on developmental rates of tobacco thrips. First instars reared on infected material took significantly longer to develop than those reared on non-infected peanut. Second instars took significantly less time to develop when reared on infected peanut versus non-infected plants. Percent mortality between *F. fusca* reared on infected versus non-infected peanuts did not differ significantly.

CHAPTER 1
INTRODUCTION

The tobacco thrips, *Frankliniella fusca* (Hinds) was first described by Hinds in 1902 as *Euthrips fuscus*. It was placed in a new genus, *Frankliniella*, by Karny in 1912 (Stannard 1968). *Frankliniella fusca* has been reported throughout most of the United States and southern Canada (Stannard 1968). It has been found on wild grasses and other flowering plants that are associated with new growth in disturbed areas (Sakimura 1963, Stannard 1968, Hobbs et al, 1993).

Frankliniella fusca occurs in a number of agronomic crops including tobacco, wheat, onion, alfalfa, barley, cowpea, oat, triticale, horseradish, and rye (Harding 1961, Gerdes 1979, McPherson et al., 1992, Buntin and Beshear 1995, Salguero-Navas et al., 1995, Sweeden et al., 1995). Peanut (*Arachis hypogaea* L.) is the crop most affected by *F. fusca* (Arant 1954, Arthur and Hyche 1959, Leuck et al., 1967, Lynch et al., 1984, Murdock et al., 1986, Herbert et al., 1991, Barbour and Brandenburg 1994) Early in the season, high populations of *F. fusca* feeding in the unfolded new buds cause scarred, malformed leaves, first described as pouts (Poos 1941, Dogger 1956). Occasionally, young peanut plants can be killed by severe feeding. Poos (1945) and other researchers reported that tobacco thrips were of a pest status important enough to warrant the exploration of control measures (Shear and Miller 1941, Leuck et al., 1967).

An examination of peanut varieties for resistance to tobacco thrips was conducted by Young et al. (1972). However, since the late 1970s, this early-season damage is often thought to be cosmetic rather than economic (Smith and Sams 1977, Tappan and Gorbet 1979 and 1981, Lynch et al., 1984, Mulder et al., 1991).

No reports of natural enemies were found in search of the literature associated with *F. fusca*. This could be partly due to the relative low status of *F. fusca* as an economic pest in peanuts.

Tobacco thrips not only cause damage to their host plants by feeding and oviposition, but more importantly, they have been shown to vector a viral pathogen that causes tomato spotted wilt disease (Sakimura 1963). Tobacco thrips also overwinter in many wild plants that serve as asymptomatic reservoir hosts important to the disease cycle of tomato spotted wilt tospovirus (TSWV) (Cho et al., 1995).

TSWV has been reported worldwide infecting 1050 plant species. Known host plants include both monocotyledons and dicotyledons in 92 families (Peters 1998). An updated list of susceptible species has been prepared and placed on the internet (http://www.spg.wau.nl/viro/research/hostlist.html) by Peters (1999).

TSWV is the type species for the genus *Tospovirus* in the family Bunyviridae (German et al., 1992, Mathews 1992). Members of this family are RNA plant viruses with membrane-bound, isometric particles. This viral disease was first described in tomato in Australia in 1919 (Franki and Hatte 1981). Presently, there are ten characterized species separated into four distinct serotypes (Xia et al., 1998). TSWV is now endemic to the southeastern United States and can be isolated from many weeds and vegetable crops, including peanuts (Culbreath et al., 1992a, 1992b, Chamberlin et al., 1992, Hobbs et al., 1993, Johnson et al., 1995, Eckel et al., 1996).

Since the first report in 1972 of TSWV in Texas, it has spread throughout southeastern United States peanut-production areas (Culbreath et al. 1990, Chamberlin et al. 1993). Symptoms on peanut include ring spotting, meristematic regions with chlorotic, stunted growth, and often the death of the plant (Culbreath et al. 1992a). During the years 1995, 1996, and 1997, losses in peanuts to TSWV in Florida were approximately five to seven million dollars (Joseph E. Funderburk, personal comm.).

Tospoviruses are vectored by seven species of thrips in a unique, persistent manner (Ullman 1997). It is generally accepted that the virus is acquired by immature thrips when they feed on infected plant material, and once infected, adult thrips can potentially remain vectors for the rest of their life (Ullman et al., 1993). Adult thrips are unable to acquire the virus, due to a midgut barrier that prevents virions from attachment and subsequent infection (Cho 1995).

Tomato spotted wilt tospovirus (TSWV) and related viruses are a worldwide problem. These viruses infect vegetable, ornamental, and, to a lesser extent, fruit crops. This disease affects both field and greenhouse production worldwide. The management of the spread of TSWV is perhaps one of the greatest global challenges facing agriculture today.

The objectives of this research were to find novel natural enemies of tobacco thrips in peanuts by several sampling techniques. In reviews of the literature, the effect of natural enemies was found to be minimal or unknown. This search for natural enemies of thrips is important because peanuts are an important source of viruliferous thrips that disperse and cause epidemics in nearby vegetable crops. The discovery of natural enemies could improve management tactics to reduce the incidence of tomato spotted wilt tospovirus.

Another aspect of this research was to examine the effects of the acquisistion of tospovirus and subsequent infection on the thrips vector. For investigations of the effects of tospovirus on another species of thrips, the western flower thrips, *Frankliniella occidentalis* (Pergande), there were no effects to reductions of life spans and fecundity (Robb 1989, DeAngelis et al., 1993, and Wijkamp et al., 1996). This latter work is important because information obtained may be applied to the management of *F. fusca*.

CHAPTER 2
DEVELOPMENT OF TOBACCO THRIPS, *FRANKLINIELLA FUSCA*, ON THREE HOSTS INCLUDING PEANUT INFECTED WITH TOMATO SPOTTED WILT TOSPOVIRUS

Introduction

Tobacco thrips, *Frankliniella fusca* (Hinds), are distributed throughout most of the eastern half of the United States and parts of the Midwest, including Oklahoma and Texas (Stannard 1968). Wild grasses and other flowering plants are natural hosts (Johnson et al., 1995, Hobbs et al., 1993). Additional hosts are several field crops including small grains, tobacco (*Nicotianna tabacum* L.), and cotton (*Gossypium hirsutum* L.) (Gerdes 1979, Burris et al., 1990, McPherson et al., 1992, Sweedon et al., 1995, Buntin and Beshear 1995, Sweedon and McLeod 1996). *Frankliniella fusca* is the most common and abundant species of thrips on peanut *Arachis hypogaea* L. in the southeastern United States. Feeding and oviposition injury on seedling peanut and cotton can be severe when large numbers of *F. fusca* are present (Poos 1941, Dogger 1956, Herbert et al., 1991, Mitchell and Smith 1991, Mulder et al., 1991). Typically, this injury on peanut is not considered economically important (Tappan and Gorbet 1981, Lynch et al., 1984, Murdock et al., 1986, Minton et al., 1991). However, certain varieties of peanut are less tolerant of injury especially when combined with other stress factors such as drought and herbicide injury (Brecke et al. 1996, Funderburk et al. 1998).

Tomato spotted wilt tospovirus (TSWV) is the type species for the genus *Tospovirus* in the family Bunyaviridae (German et al., 1992). This disease was first described in tomato in Australia in 1919 (Franki and Hatte 1981). There are ten characterized species of tospoviruses separated into four distinct serotypes (Xia et al., 1998).

Tospoviruses are vectored by seven species of thrips in a unique, persistent manner (Ullman et al., 1997). The virus is acquired by immature thrips feeding on infected plants (Ullman et al., 1993). Several species of thrips are important vectors of tomato spotted wilt tospovirus in the southeastern United States, including *F. fusca* and *F. occidentalis* (Pergande) (Sakimura 1963). Other thrips species have been found in peanut but apparently, these are not important to the spread of the virus.

Some of the symptoms of tomato spotted wilt tospovirus on peanut include ring spotting, yellowing, stunting, and often death of the plant (Culbreath et al. 1992). Symptoms are similar on solanaceous crops, with fruit from infected plants unmarketable.

There is little information available of the effects of tomato spotted wilt tospovirus on the biology of *F. fusca*. The effects of this virus group on the biology of *F. occidentalis* have been studied, including developmental time, fecundity, and mortality (Robb 1989, DeAngelis et al. 1993, Wijkamp et al. 1996). In such studies, it was difficult to ascertain if the observed differential biology of thrips feeding on infected or non-infected hosts resulted directly from viral acquisition, or from changes in host plant chemistry due to infection.

While viral expression may change plant chemistry in a variety of ways, the most consistent aspect related to insect performance (growth, development rates, fecundity) is the availability of dietary nitrogen (Slansky and Scriber 1985). Feeding and development of thrips species are affected by foliar nitrogen in a variety of host species (Mollema and Cole 1996).

Similarly, there is little information to determine the suitability of different crop hosts on the developmental biology of *F. fusca*. Yet, many crops are suitable hosts for *F. fusca* in the eastern U.S., and this thrips species is an important vector of tomato spotted wilt tospovirus in peanut and in solanaceous crops (Culbreath et al. 1990, Hobbs et al. 1993, Johnson et al., 1995). The objectives of this study were to compare development and larval mortality of *F. fusca* on peanut, cotton, and tomato and to determine the effects of TSWV on development, larval mortality, and fecundity of *F. fusca*. An additional objective of this research was to assess potential nutritional effects of infected peanut plants on the biology of *F. fusca* by determination of the nutrient (nitrogen) status of infected and non-infected plant hosts.

Materials and Methods

Host Developmental Study

Colonies of *F. fusca* were maintained in a greenhouse with an average temperature of 28°C. These colonies were originally established with adults collected from peanut leaves and flowers at the North Florida Research Center in Marianna and the Green Acres research farm in Gainesville. Field-collected adults were placed in plexiglass cages (56 cm by 32 cm by 48 cm) that contained 3-week old peanut plants. These plants were replaced as needed with new plants of the same age. Additionally, field-collected adults of *F. fusca* were allowed to oviposit on peanut leaves placed in 33-ml diet cups, and 20 to 30 larvae from these cups were added to colonies every three weeks.

Newly emerged, first instars from the colony were confined in modified Munger (1942) leaf cages for easy observation and to reduce handling effects on their development. The base of each cage consisted of an 8.5 cm by 11 cm slab of 0.65 cm plexiglas and a 3 cm white cardboard disk. Individual leaves from one of three plants: tomato (cv. 'Bonnie Best'), cotton (cv. 'Deltapine 20'), or peanut (cv. 'Tamrun 88'), were placed on the cardboard disk followed by an arena slab with a 1 cm diameter hole. Individual thrips were introduced with the aid of a cotton swab. The separate slabs of each cage were secured with binder clips to complete the cage. Distilled water was dripped between the sandwich layers to the cardboard disk to prevent leaf wilt. Leaves were changed every 2 days. Cages were maintained in a growth chamber set at 27 ± 2^{o}C with a light/dark regime of 14/10 hours. Relative humidity of the chamber was maintained at approximately 55%. Thrips were examined once every 24 hours to monitor development until the adult stage. Effects of crop host on developmental time of each life stage and from first instar larva to adult were evaluated by analysis of variance and subsequent orthogonal treatment comparisons (SAS 1990). A chi-square test was used to compare mortality between hosts (Steel and Torrie 1959).

Viral Infection on Developmental Rates

Newly hatched, individual thrips were added to modified Munger (1942) cages with leaves of approximately the same age from non-infected and tospovirus infected peanut plants. The isolate of tomato spotted wilt tospovirus was obtained from infected peanut plants growing in the field at the North Florida Research and Education Center, Marianna. Potential nutritional effects on developmental times were reduced by limiting acquisition time on infected leaf material to 24 hours; thereafter, leaves from all cages were replaced daily with healthy leaves. Thrips were observed every 24 hours until death. Individuals that survived to adult from both treatments were allowed to feed on peanut leaves from non-infected plants. All leaves were frozen at -70°C until an enzyme-linked immunosorbent assay (ELISA) procedure (Agdia, Elkhart, IN) was performed to confirm presence or absence of tomato spotted wilt tospovirus. Effects of viral infection on developmental time and mortality of the thrips were evaluated using statistical procedures described previously (SAS 1990).

Leaf Nitrogen Content

The nitrogen content of leaves from each treatment was evaluated to determine if nutritional factors were involved in any observed differences in developmental time or mortality of infected or non-infected thrips. Leaflets from six severely infected and six control plants were collected from the field and immediately frozen in liquid nitrogen to arrest enzyme activity. Samples were stored frozen and lyophilized prior to analysis (Brodbeck et al., 1996). Nitrogen concentrations were determined by analysis of samples in triplicate by combustion technique on a Perkin Elmer 2400 CHNS Analyzer (Brodbeck et al. 1996). Treatment effects were analyzed by a t-test (SAS 1990). Three voucher specimens from the *F. fusca* colony were deposited at the Florida Department of Agriculture and Consumer Services, Division of Plant Industries, Florida State Collection of Arthropods, Gainesville, Florida.

Results

Host Developmental Study

Developmental times of larvae and pupae of *F. fusca* on peanut, cotton, and tomato are given in Table 2.1. First and second instars of *F. fusca* thrips developed significantly slower on tomato than on peanut and cotton (F = 5.1 and 4.2, respectively; df = 1, 42; $P < 0.05$). Development of first and second instars was similar on peanut and cotton (F = 1.1 and 0.0, respectively; df = 1, 42). Developmental times for pupae I and pupae II were statistically similar on each crop host (F = 2.0 and 1.4; df = 2, 34). Total time of development was significantly longer for thrips feeding on tomato than for thrips feeding on peanut and cotton (F = 5.6; df = 1, 34; $P < 0.05$). Total time of development was similar for thrips feeding on peanut and cotton (F = 0.0; df = 1, 34). Mortality of *F. fusca* on peanut, cotton, and tomato was 4, 12, and 42%, respectively (Table 2.1). These differences in larval mortality were highly significant ($x^2 = 11.9$, df = 2, $P < 0.01$).

Table 2.1. Mean developmental times (days) of first and second instar larvae and pupae of *Frankliniella fusca* (±SEM) and larval mortality on three hosts at 25°C.

		I Larva	II Larva	Pupa I	Pupa II	Total	% Mortality
Host	N	Mean^x ± SEM^y	Mean^x ± SEM^y	Mean^x ± SEM^y	Mean^x ± SEM^y	Mean^x ± SEM^y	
Peanut	25	1.4 ± 0.17^a	4.7 ± 0.32^a	1.6 ± 0.27^a	2.6 ± 0.17^a	10.3 ± 0.43^a	4
Cotton	26	2.0 ± 0.25^a	4.6 ± 0.38^a	1.4 ± 0.13^a	2.5 ± 0.16^a	10.5 ± 0.33^a	12
Tomato	24	2.9 ± 0.66^b	6.2 ± 1.04^b	1.0 ± 0.10^a	3.0 ± 0.30^a	11.9 ± 0.40^b	42

[x] Means followed by different letters (within column) are signifcantly different using orthogonal contrasts at $P < 0.05$ (SAS Institute, 1990).

[y] Standard error of mean.

Leaf Nitrogen Analysis

Percent nitrogen content in peanut leaves was not affected by TSWV infection ($t = 0.28$, df = 10). Mean percent nitrogen levels (±SEM) in leaves from non-infected and infected peanut plants were 2.90 (±0.22) and 2.98 (±0.15), respectively. Percent crude protein levels in non-infected and infected leaves was 18.13% and 18.63%.

Viral Infection on Developmental Rates

Developmental rates of *F. fusca* on healthy and infected peanut leaves are presented in Table 2.2. All infected and non-infected larvae developed to second instar in 1 or 2 days. Even so, mean time of development of first instars was significantly slower for infected than non-infected larvae ($F = 8.2$ df = 1, 83; $P < 0.01$) Conversely, mean time of development of second instars was significantly slower for non-infected than infected larvae ($F = 10.1$; df = 1, 83; $P < 0.01$). Overall time of development of both larval stages was similar for both infected and non-infected larvae ($F = 0.1$ and 1.6, respectively; df =1, 80). Mortality of larvae was < 4% for infected and non-infected larvae (Table 2.3), and there was no significant difference ($x^2 = -1.6$; df = 1). The ELISA proceedure indicated that 86% of the adults developed from larvae that fed on leaves from infected plants for 24 hours were able to transmit tomato spotted wilt tospovirus. The ELISA procedures were negative for all non-infected larvae.

Table 2.2: Chi-square analysis of mortality percentages for *Frankliniella fusca* on infected and healthy peanut.

Host	Alive	Dead	Total	p^y	pA^z
Peanut	24	1	25	0.96	23.04
Cotton	23	3	26	0.88	20.24
Tomato	14	10	24	0.58	8.12

$x^2 = (P < 0.01)$.

[y]Proportion of total alive.

[z]Number alive multiplied by p.

Table 2.3. Mean developmental times (days) of larvae and pupae of *Frankliniella fusca* (±SEM) nfected or non-infected peanut plants at 25°C.

Treatment	N	Larva I Mean[x] ± SEM[y]	Larva II Mean[x] ± SEM[y]	Pupa I Mean[x] ± SEM[y]	Pupa II Mean[x] ± SEM[y]	Total Mean[x] ± SEM[y]	% Mortality
Non-infected	34	1.1 ± 0.1^{a}	4.2 ± 0.1^{a}	1.0 ± 0.0^{a}	2.0 ± 0.0^{a}	8.3 ± 0.1^{a}	2.9
TSWV-infected	51	1.4 ± 0.1^{b}	3.6 ± 0.1^{b}	1.0 ± 0.0^{a}	2.1 ± 0.1^{a}	8.1 ± 0.2^{a}	3.9

[x] Means followed by different letters (within column) are signifcantly different using ANOVA at $P < 0.05$ (SAS Institute, 1990).

[y] Standard error of mean.

Discussion

The developmental rates of *F. fusca* on peanut in modified Munger cages at 25°C was shown to be similar to results obtained by Lowry and co-workers (1992) on peanut variety (cv. 'Florunner'). They found *F. fusca* developed in 1.1, 4.7, 1.1, 2.0 days for first, second instars, pupa I, and pupa II, respectively. Several varieties of peanut have displayed moderate resistance to *F. fusca* (Kinzer et al., 1973). Previous research performed on the development of *F. fusca* on cotton seedlings had a total development time of 14.9 days (Watts 1934). Watts gave no indication of the cultivar of cotton used in his studies.

Tomato appears not to be an ideal host for *F. fusca*. Larval developmental times on tomato were significantly slower than on peanut and cotton. Mortality rates were also much higher (44%). All instars were often observed to spend more time wandering in the cages with tomato leaves than with the other two hosts. On tomato, first instars were often tangled or trapped by trichomes covering leaf surfaces. *Frankliniella fusca* is typically the most abundant species of thrips found on peanut and cotton but not on tomato. *Frankliniella fusca* collected from tomato in the field are usually adults found in the blooms (Salguero-Navas et al., 1995). However, adults have been shown to feed on seedling tomato when given no alternative. Tomato is one crop adversely affected by tomato spotted wilt virus tospovirus. Adult *F. fusca* can acquire the virus while developing on peanut and then vector the virus as they probe other plants, including tomato.

Developmental rates of *F. fusca* on 'Tamrun 88 peanut at 25^{o}C in the viral infection experiment was very similar to developmental rates at the same temperature obtained by Lowry et al. (1992) on 'Florunner' peanut. Developmental times for larvae I, larvae II, pupae I, and pupae II in their study were 1.1, 4.7, 1.1, and 2.0 days, respectively. They reported no differences in the duration of any stage of *F. fusca* when the larvae were fed on folded peanut terminals or unfolded peanut leaflets. Non-infected larvae and pupae on peanut developed about 15% faster in the experiment on viral infection than in the host development experiment. Differences in total time of larval and pupal development on peanut between our two experiments undoubtedly are due to slightly different conditions of handling time or temperature. Watts (1934) reported developmental times of 2.28, 3.54, 1.11 and 2.63 days for larvae I, larvae II, pupae I, and pupae II respectively. The cotton cultivar used in the experiment and the temperatures were not given, however, the developmental times compared favorably with developmental time for *F. fusca* at 25^{0}C on 'Deltapine 20' cotton in our experiment. Mortality of larvae and pupae of *F. fusca* that developed on cotton and peanut was very low in this study. In our studies, 'Tamrun 88' peanut and 'Deltapine 20' cotton were equally good hosts for development and survival of *F. fusca*. This may be typical for commercial cultivars grown in our geographic region since commercial cultivars of both cotton and peanut can be heavily injured by *F. fusca* (Lynch et al., 1984, Watson 1965). Genotypes of cotton and peanut with resistance to thrips have been identified, but thrips-resistant cultivars have not been released.

Antibiosis has been reported as a resistance mechanism to *F. fusca* in some peanut breeding lines. Variation in susceptibility of cotton cultivars to thrips have been shown in association with the level of secondary plant metabolites such as gossypol, tannins, and flavenoids (Gopichandran et al., 1992) and with morphological characters including pubescence (Quisenberry and Rummel 1979). Time of development and mortality of *F. fusca* were increased by letting them feed on tomato in this study. Larvae were observed to spend more time wandering on tomato than on peanut or cotton. Often, first instars were tangled of trapped by glandular trichomes of commercial tomato cultivars. Wild *Lycopersicon* species confer resistance to *F. occidentalis*, either as a result of less feeding or toxicity. Kumar et al. (1995a) determined that differences in transmission of virus were due to differential feeding by the thrips after landing. Larvae of *F. occidentalis* avoided tomato leaves with dense glandular trichomes (Kumar et al., 1995b).

Under field conditions, several species of *Frankliniella* occur on tomato in the southeastern U.S., mostly on flowers and small fruit (Salguero Navas et al. 1991a). Populations of adult *F. fusca* occur only in very low densities, and the species was not reported to reproduce successfully (Salguero Navas et al., 1991b). However, populations of *F. fusca* that developed on peanut infected with tospovirus have been shown to infect tomato plants after probing the leaves (D. Chellemi, J. Stavisky, J. E. Funderburk, and H. Dankers, unpublished). Adults of *F. fusca* that migrate from peanut to tomato have been implicated in causing epidemics of tomato spotted wilt tospovirus in commercial tomato (J. E. F., personal observation). This vector certainly is important in causing tospovirus epidemics in other solanaceous crops in our region (Johnson et al., 1995).

Some effects of infection by TSWV on *F. fusca* appear as modifications to rates of larval development. First instars develop slower when placed on infected leaves. However, infected individuals developed faster in the second larval stage. A similar result was found for *F. occidentalis* when reared on TSWV-infected tissue (Robb 1989). TSWV seems to have little effect on the two pupal stages of *F. fusca*. Additionally, there appears to be no viral effect on mortality rates of *F. fusca*. Studies investigating the effect of tospsovirus on *F. occidentalis*, in which either first or both first and second instars were exposed (Robb 1989, DeAngelis et al. 1993), showed an increased mortality rate. However, Wijkamp and co-workers (1996) found no effects of tospovirus infection on developmental rates and survival for *F. occidentalis* reared on infected *Datura stramonium* L. In their study, a short viral acquisition time of 6 hours was chosen to reduce potential effects of the nutritional quality of infected plants on thrips physiology. There was no difference in the nitrogen levels of tospovirus-infected and healthy peanut leaves in our study.

The percentage of successful acquisition and transmission of virus by *F. fusca* (86%) was much higher than the 26% reported by Sakimura (1963). This large difference could have been due to the increased sensitivity of ELISA tests used presently. More importantly, the host plants used by Sakimura (1963) to test *F. fusca* might not have been preferred hosts. Additionally, the viral strain could be a potential source of variation associated with acquisition and subsequent vectoring.

CHAPTER 3
NATURAL ENEMIES OF *FRANKLINIELLA FUSCA*

Introduction

The majority of research associated with natural enemies of field populations of pest species of thrips is the examination of minute pirate bugs, Anthocoridae, and predatory mites (Phytoseiidae) (Stoltz and Stern 1978, Isenhour and Yeargan 1981, Letourneau and Altieri 1983, Etienne et al., 1990, Shipp and Whitfield 1991, Trjapitzin 1995). Additionally, several researchers report ants, spiders, nematodes, and parasitic Hymenoptera as natural enemies of thrips (Wilson and Cooley 1972, Beshear 1974, Gabarra et. al., 1988, Loomans et. al., 1995, Lewis 1973). As species of thrips that are vectors of tospovirus become more important, undoubtedly more time, money, and effort will be spent to search for natural enemies as tools for pest-management programs.

The evaluation of fungal pathogens to control thrips has been limited mainly to western flower thrips, *Frankliniella occidentalis*, that occur in greenhouses and pear thrips, *Taeniothrips inconsequens* (Brownbridge et al., 1993, Brownbridge 1995). Three fungal pathogens have been identified as potentially important parasites of thrips: *Beauveria bassiana* (Bals.), *Metarhizium anisopliae* (Metsch.), and *Verticillium lecanii* Zimm. (Skinner et. al., 1991, Brownbridge 1995).

Nematodes in the genus *Thripinema* are obligate parasites of Thysanoptera. Siddiqi (1986) created the genus based on four previous species descriptions. Currently, there are five described species (Tipping et al., 1998).

Materials and Methods

The search to discover undescribed natural enemies of *F. fusca* involved weekly sampling and the development of several types of traps. During the summers of 1995, 1996, and 1997, plots of peanut *Arachis hypogaea* L. were sampled by the random collection of a single, newly unfolded leaflet, and, when available, a single flower. An individual sample was collected within a single approximately 0.5 hectare plot divided into six equal-sized blocks. Each block was randomly sampled five times per week with a total of thirty per sample date. Each plot was surrounded by plots of peanuts of different ages and varieties. Peanuts were planted 13 May 1995, 8 May 1996, and 28 May 1997. Sampling began approximately three weeks after planting and continued each week until peanut harvest. Collected plant material was placed in 33-ml plastic diet cups maintained in an environmental chamber set at a 14/10 hour photoperiod at 25°C.

All immature thrips collected on peanut foliage and flowers were allowed to develop to the adult stage and subsequently used to check for pathogens or parasites. Adults were also examined for parasites and pathogens.

Yellow sticky-card traps, one cm^2, were pinned to peanut plants in the field to collect parasitic Hymenoptera. Plastic shell-vials (35 ml) were filled a 3% soap-water solution and placed in the field (Figure 3.1). Vials were secured to flat wooden stakes with rubber bands at the height of the tallest peanut leaves. A yellow plastic disk was floated on the surface of the solution. Also attached to the stake was a flap made of yellow plastic that served as protection against overflow during rain storms. Contents of both types of traps were collected and evaluated weekly. Adult thrips were collected and dissected to determine percentages parasitism of a newly discovered nematode.

Results

The results of sampling peanut in Marianna, Florida for the years 1995, 1996, and 1997 are presented in Figures 3.2, 3.3, and 3.4 respectively. The population trend of both the immatures and adult thrips tended to be higher early in the season for all three years, with lowers numbers at harvest. Examination of the insects captured on the small yellow sticky traps proved difficult. Specimens could not be removed from the plastic without damage, making proper identification to species impossible. Also, many *F. fusca* were found in these traps. During the first week of September 1995, examination of the contents of a water trap showed the presence of nematodes within the body cavity of many individual *F. fusca*. In comparison to descriptions of known species of *Thripinema*, this species was considered as a new, undescribed nematode. We named it *Thripinema fuscum* after its host *F. fusca* (see Chapter 4). Percentage of parasitism was determined by dissecting live

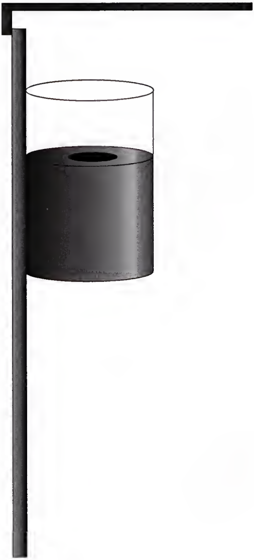


Figure 3.1: Vial trap.

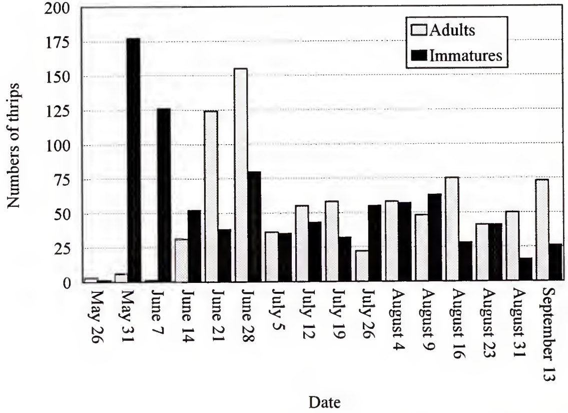


Figure 3.2: Number of thrips per sample of *Frankliniella fusca* stages collected in peanut, *Arachis hypogaea* during summer 1995, Marianna, Florida.

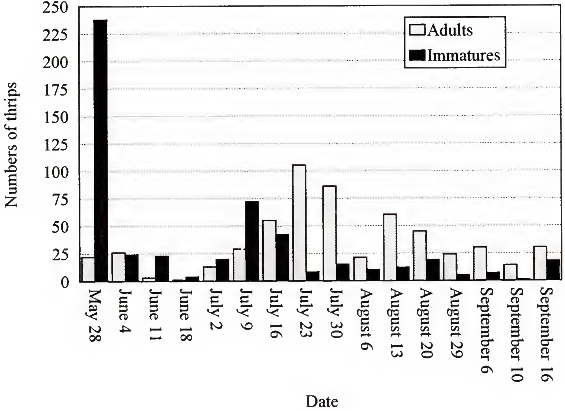


Figure 3.3: Number thrips per sample of *Frankliniella fusca* stages collected in peanut, *Arachis hypogaea* during summer 1996, Marianna, Florida.

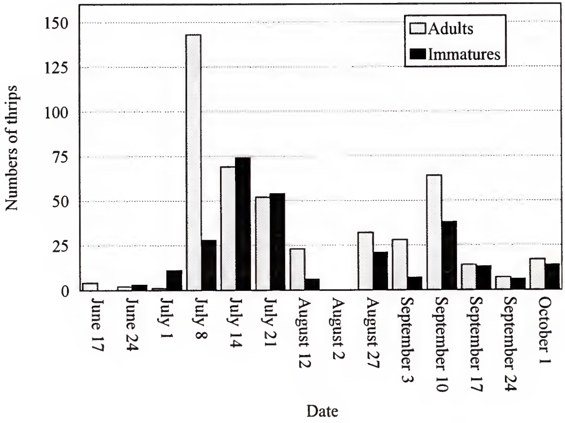


Figure 3.4: Number of thrips per sample of *Frankliniella fusca* stages collected in peanut, *Arachis hypogaea* during summer 1997, Marianna, Florida.

thrips that were collected weekly. The percentage of parasitized thrips are presented in Tables 3.1, 3.2, and 3.3 for the three years of data collected in Marianna, Florida.

Discussion

In this study we surveyed naturally occurring populations of *F. fusca* on peanut. After sampling with a variety of techniques including collection of plant material, sticky traps, and soap water traps, we detected a new important natural enemy of *F. fusca*. A nematode belonging the genus *Thripinema* was described as *T. fuscum* (Tipping et al. 1998). After examination of *F. fusca* found in peanut throughout the southeastern U.S., we feel this organism is likely the most common natural enemy of populations of *F. fusca* that occur on peanut. Extensive surveys of populations of *F. fusca* on native hosts have not been completed. The reduction of numbers of *F. fusca* in peanut as the season progresses has been long noticed but not understood. We feel this population reduction is the result of parasitism provided by *T. fuscum*. We have found this parasite in regions of peanut production in north and central Florida as well as parts of Georgia and South Carolina.

One of the most important aspects of any integrated-pest-management strategy is to identify natural enemies of the problematic species. Careful examination of the biology of the pest species can often lead to more cost efficient methods of control by utilizing natural enemies. The discovery of *T. fuscum* could provide future researchers with a tool to control outbreaks of *F. fusca*, and to ultimately reduce the severity of tospovirus outbreaks.

Table 3.1: Percentage of parasitism of *Frankliniella fusca* by *Thripinema fuscum* in peanut from Marianna, Florida, 1995 (average = 49.8).

Date	Parasitism (%)
September 13	43.5
September 20	39.1
October 4	66.7

Table 3.2: Percentage of parasitism of *Frankliniella fusca* by *Thripinema fuscum* in peanut from Marianna, Florida, 1996 (average = 40.8).

Date	Parasitism (%)
July 16	47.6
July 23	43.4
July 30	37.5
August 6	37.1
August 13	51.9
August 20	46.9
August 29	33.3
September 6	62.9
September 10	41.3
September 16	64.1

Table 3.3: Percentage of parasitism of *Frankliniella fusca* by *Thripinema fuscum* in peanut from Marianna, Florida, 1995 (average = 47.8).

Date	Parasitism (%)
July 8	12.6
July 14	21.3
July 21	53.1
August 12	56.2
August 22	41.7
August 27	58.5
September 3	56.2
September 10	70.6
September 17	40.4
September 24	37.5
October 1	30.0

CHAPTER 4
THRIPINEMA FUSCUM N. SP. (TYLENCHIDA: ALLANTONEMATIDAE), A PARASITE OF THE TOBACCO THRIPS, *FRANKLINIELLA FUSCA* (THYSANOPTERA)

Introduction

Nematodes infesting Thysanoptera were first recorded from Europe in *Thrips physophus* L. (Uzel, 1895). Russell (1912) found a nematode inhabiting the bean thrips, *Heliothrips fasciatus* (L.), in California but did not describe it. Sharga (1932) described a nematode from *Aptinothrips rufus* (Gmelin) in England as *Tylenchus aptini*. Lysaght (1936) proposed the taxon *Anguillulina aptini* for this species, and a year later published an ecological study of the nematode including seasonal abundance and life cycle (Lysaght 1937). Wachek (1955) transferred *A. aptini* to the genus *Howardula*. Nickle and Wood (1964) reported *Howardula aptini* infecting two species of blueberry thrips, *Frankliniella vaccinii* Morgan and *Taeniothrips vaccinophilus* Hood. Reddy et al. (1982) reported *Howardula aptini* infecting *Megaluriothrips* sp.

Siddiqi (1986) created a new genus, *Thripinema*, based on host range and the oval shape of the parasitic female. His revision of the genus included renaming the nematodes described by Sharga (1932), Nickle and Wood (1964), and Reddy et al. (1982) as *T. aptini*, *T. nicklewoodi*, and *T. reniraoi*, respectively. More recently, Chizov et al. (1995) described *Thripinema khrustalevi* from two hosts, *Thrips trehernei* Prisner and *T. physophus* L.

During the summers of 1995, 1996, and 1997, we conducted a survey of the natural enemies of tobacco thrips, *Frankliniella fusca* (Hinds), found on Florunner peanut (*Arachis hypogaea* L.) growing at the North Florida Research and Education Center field station in Marianna, Florida. Several nematodes were discovered within the abdominal cavity of an adult female *F. fusca*. After collection of additional specimens, we concluded that the nematode represented a new species described as *Thripinema fuscum* Tipping and Nguyen (Tipping et al. 1998). The new species is named after the host, *F. fusca*.

Materials and Methods

Collection of Nematodes

Collections of peanut flowers began September 1995 until the peanut harvest in October and again in 1996 and 1997 from July to the October peanut harvest. Percent infection of thrips was estimated from the weekly collections thrips from 35 randomly selected flowers. Flowers were gently opened with forceps while submerged in a 5% formalin solution. All stages of thrips present were dissected. Parasitic and unmated reproductive-stage females, eggs, adult males, and all juvenile stages were found. Infective stage females were obtained when peanut flowers were dissected in a small dish of distilled water and viewed through a stereo scope (Wild M3) at 35X.

Light microscopy preparation

Nematodes of all life stages were dissected from thrips and also collected from peanut flowers were transferred to warm lactophenol for 3 days before they were mounted in glycerin on glass slides (Coler and Nguyen 1994). Measurements are in μm and given as means ± standard deviations followed by ranges.

Systematics

Thripinema fuscum Tipping and Nguyen, 1997 (Figs. 4.1, 4.2).

Description

Holotype (male): Length = 295.5 μm; width = 13.6 μm; a = 21.6; c = 9.3; stylet length = 10.6 μm; spicule length = 12.3 μm; gubernaculum length = 5.5 μm; tail length = 31.8 μm.

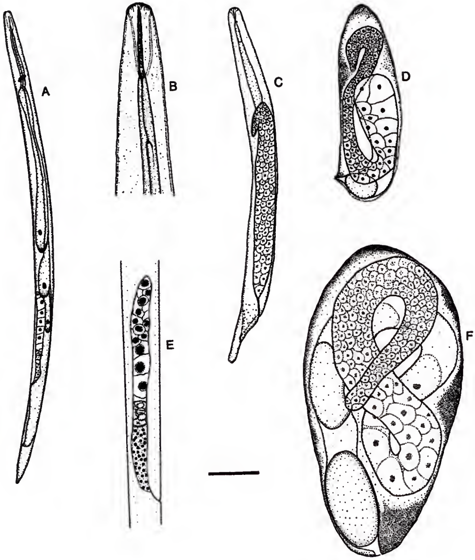
Males (n = 11): Length = 302.1 ± 27 (254-333); greatest width = 14.9 ± 1.8 (12.1-16.7); a = 20.4 ± 2.0 (17.5-23.5); c = 9.3 ± 0.7 (8.4-10.2); stylet length = 11.4 ± 2.0 (9.0-15.2); spicule length = 12.9 ± 1.5 (10.3-15.2); gubernaculum length = 6 ± 1 (3.6-7.0); tail length = 32.5 ± 3.5 (27.3-37.8).

Parasitic females (n = 11): Length = 152.3 ± 26.8 (121.0-186.0); width = 72.8 ± 9.7 (59.0-94.0); a = 2.1 ± 0.3 (1.6-2.5).

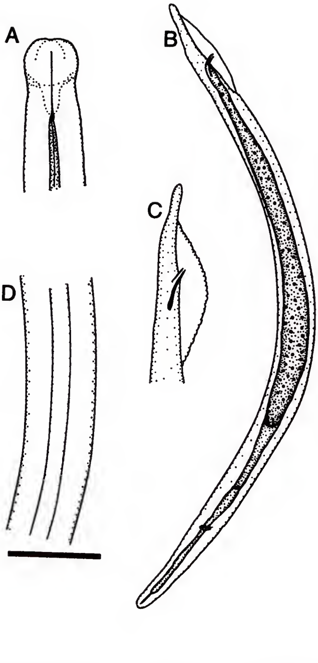
Eggs (n = 13): Length = 53.6 ± 9.6 (42.4-71.2); width = 26.8 ± 5.8 (16.7-37.9).

Allotype (infective female): Length = 274 μm; width = 12.1 μm; a = 22.6; b = 1.5; c = 11.9; esophagus length = 178 μm; stylet length = 12.7 μm; V% = 84; tail length = 23 μm.

Infective females (n=16): Length = 257.4 ± 14.6 (227.0-279.0); width = 12.2 ± 1.1 (10.0-13.6); a = 21.4 ± 2.2 (17.0-25.5); b = 1.5 ± 0.1 (1.3-1.7); c = 11.6 ± 1.0 (10.5-13.0); esophagus length =



4.1: *Thripinema fuscum* n. sp. female. A) Infective female. B) Anterior region of an infective female. C, D, F) Progressive enlargement of parasitic females. E) Gonad of infective female. Scale bar 26μm for A, C, D, F; 12 μm for B, E.



4.2: *Thripinema fuscum* n. sp. male. A) Anterior region of male. B) Young male exhibiting slight dorsal curve. C) Bursa. D) Lateral field of male. Scale bar 14 µm for A, C, D; 30 µm for B.

167.2 ± 13.6 (139-189); stylet length = 11.5 ± 1.3 (9.1-13.6); V% = 81.8 ± 1.7 (78.0-84.0); tail length = 22.4 ± 2.2 (19.7-24.2).

Parasitic females: Different stages of parasitic females found in thrips, ranged from slightly swollen young females (Fig. 4.1C) to fully swollen older mature females (Figs. 4.1D, F). Body of mature female oval or elliptical. Stylet and esophagus indistinct or not observed in mature females but distinct in younger females. Vulva subterminal. Occasionally, part of uterus protruding from vulva. Ovary long, convoluted, with two or three flexures. Uterus large, usually containing a single egg (Figs. 4.1D, F).

Infective females: Body nearly straight or slightly curved ventrally when relaxed (Fig. 4.1A). Lip region moderately sclerotized, continuous with body, sometimes slightly offset. Lateral fields with two incisures forming a band occupying up to 40% of body width. Stylet about three times as long as head width (Fig. 4.1B), without knobs but with slight basal thickening. Stylet lumen wide. Dorso-esophageal gland orifice more than one stylet length posterior to stylet base. Esophagus long, posterior end reaching beyond mid-body (Fig 4.1A). Anterior part of esophagus thin, less than 40 μm long. then reduced posteriorly. Esophageal glands well developed, long, extending two-thirds length of body with nuclei usually distinct. Nerve ring located near base anterior part of esophagus. Excretory pore not observed. Ovary single, prodelphic. Uterus well developed, containing sperm in mature females (Fig. 4.1E). Vulva usually one or more tail lengths anterior to anus. Tail tapering posteriorly to a rounded or conical tip, sometimes with a short hyaline portion.

Free-living males: Body curved dorsally when relaxed (Fig. 4.2B). Lateral fields with two incisures forming a smooth band (Fig. 4.2D) occupying one-fifth to one-fourth of body width. Lip region usually slightly swollen, occasionally distinctly offset, moderately sclerotized. Stylet present (Fig. 4.2A), very thin, nearly indistinguishable after fixation. Stylet base slightly thickened. Esophagus degenerate, indistinct. Testis well developed, usually occupying up to one half of body cavity diameter at mid-body and containing numerous sperm throughout its entire length. Spicules paired, thin. Gubernaculum straight or slightly curved with posterior tip rounded, somewhat curved dorsally, well cuticularized. Bursa large, curved with crenate margins (Fig. 4.2C).

Type Host and Locality

Tobacco thrips, *Frankliniella fusca* (Hinds), collected from peanut (*Arachis hypogaea* L.), Jackson County, Marianna, Florida, USA.

Type Specimens

Holotype (male) and allotype (infective female): Deposited in the U. S. Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland, USA.

Paratypes: Deposited in the USDANC, and the Florida Department of Agriculture and Consumer Services, Division of Plant Industry Nematode Collection, Gainesville, FL, USA.

Diagnosis

Male large (averaging 302 µm), body curved dorsally with thin stylet present. Spicule averaging 12.9 µm. Parasitic females oval, averaging 152*73 µm. Stylet not observed. Eggs large, 54*27 µm. Infective female averaging 257 µm, stylet base without knobs but slightly thickened, *c* = 11.5.

Relationships

Thripinema fuscum n. sp. can be distinguished from all other species of *Thripinema* spp. by the male possessing a stylet and a dorsally curved body. Further, *T. fuscum* n. sp. differs from *T. reniraoi* by the *c* ratio (11.6 vs. 9.9) and large eggs (55*27 µm vs. 30*28 µm); from *T. nicklewoodi* by parasitic female size (152*73 µm vs. 210*56 µm), ratio *a* (2.1 vs. 3.8), large eggs (55*27 µm vs. 35*18 µm); from *T. khrustalevi* by parasitic female size (152*73 µm vs. 250*120 mm) and stylet length of infective females (11.5 vs. 13-15 µm); from *T. aptini* by the parasitic female size (152*73 µm vs. 291*75 µm) and male tail length (27-38 µm vs. 21-27 µm). The following key will assist in identification of known species belonging to *Thripinema*. Both male and female specimens are needed for identification.

Key to Species of the Genus *Thripinema*

1. Caudal alae reaching tail tip (peloderan)....................................2
   Caudal alae not reaching tail tip (leptoderan)..........................3

2. Parasitic female spindle-shaped or bean-shaped with tail ending in a sharp point; V% about 84........*T. nicklewoodi*
   Parasitic female oval or spherical, vulva terminal.................
   ....*T. khrustalevi*

3. Stylet present in male, male body curved dorsally.............
....*T. fuscum n. sp.*
Stylet absent in male, male body not curved dorsally........4

4. Parasitic female small, 150-225 mμ long................*T. reniraoi*
Parasitic female large, 266-316 mμ long....................*T. aptini*

Biology

Infective stage females of *Thripinema fuscum* n. sp. can be collected by gently swirling peanut flowers in a dish of distilled water. After entering the host thrips by penetration through the intersegmental membrane of the coxal cavities, females change into the characteristic swollen shape for the genus. The ovary increases in length with several flexures. Adult thrips and rarely, second instars, were parasitized. Pupae were not hosts for *T. fuscum*. The ovaries of the parasitized thrips are reduced and eggs are absent. Generally, only one parasitic female per host is present but occasionally two or three have been observed. Although up to 175 immature nematodes were dissected from one insect, usually 40 to 50 were normally present. After development within the host, free-living males and females exit thrips via the anus as reported by Lysaght (1937) for *T. aptini* and Nickle and Wood (1964) for *T. nicklewoodi*. Mating of *T. fuscum* probably occurs in the peanut flowers.

In Florida, *F. fusca* is found to develop in wheat (*Triticum aestivum* L.) and flowers of wild radish (*Raphanus raphanistrum* L.)

during January through March. *Thripinema fuscum* has been found parasitizing tobacco thrips collected from these winter host plants.

Western flower thrips, *Frankliniella occidentalis* (Pergande) are occasionally infected with *T. fuscum*. *Thripinema nicklewoodi* has been shown to readily parasitize *F. occidentalis* (Wilson and Cooley 1972, Greene and Parrella 1995). Nickle and Wood (1964) reported that pupae of *Frankliniella vaccinii* and *Taeniothrips vaccinophilus* were also parasitized by *T. nicklewoodi*.

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BIOGRAPHICAL SKETCH

Christopher Tipping was born in Upper Darby, Pennsylvania and raised in Newark, Delaware. He completed his B. Sc. in entomology and plant pathology at the University of Delaware in 1986. During this time he completed and published an undergraduate honors research project on the genetics of lab colonies of the European corn borer, *Ostrinia nubilalis* (Hubner). He worked for the USDA-ARS Beneficial Insects Research Lab (BIRL), Newark, DE, with the biocontrol lab for gypsy moth, *Lymantria dispar* (L.) control, where he evaluated the effects of recently imported parasitic species on host competition.

He was employed as a research assistant in Department of Animal Science at the University of Delaware working with ruminant nutrition and microbial associations with silage from 1986 to 1989. He continued to work in entomology during which time he documented oviposition behavior of *Culex pipiens* L. and *Cx. restuans* Theobald.

After employment with the E. I. duPont de Nemours and Company insect control group in 1992, Chris was enrolled in the master of science degree program at the University of Delaware. His project was the systematic examination of the Proturan genus *Eosentomon* from the interior highlands of Arkansas. During this time he also was involved in the discovery and description of new species of Diplura, Thysanura, and Symphyla.

After the description of several new species of *Eosentomon* and the completion of his M.S. degree, Chris began his Ph.D. program at the department of entomology and nematology of the University of Florida in January 1995. His Ph.D. project involved the search for novel natural enemies of tobacco thrips, *Frankliniella fusca* (Hinds), as well as the effects of tomato spotted wilt tospovirus on the biology of *F. fusca*.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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